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The microflora of the alimentary canal of *Achipteria coleoptrata* (Acarina, Oribatei)

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Contents

1. Introduction

The results of preliminary studies on the occurrence, composition and role of the microflora of the alimentary canal of some mites (Prusinkiewicz, Stefaniak and Seniczak 1975) show that:

– the microflora of the mite species under study differs from that of their habitat,
– the particular species which are xylophages [e.g. *Phthiracarus piger* (Scop.) and *Tropacarus carinatus* (C. L. Koch)], lichenophages [*Trhypochthonius cladonicola* (Willm.)], and polyphages [*Oppia obsoleta* (Paoli) and *Tectocepheus velatus* (Mich.)] differ with regard to the associations of microflora present in the alimentary canal.
– the microflora of the alimentary canal of monophages is undoubtedly influenced by the kind of food they take in.

These data based exclusively on investigations of adult forms require confirmation and more extensive work

The fact that juvenile stages of mites were considered to be more important in the transformations of organic substances in soil than adult individuals (Berthet 1963; Webb 1970) induced us to undertake detailed microbiological studies, which, in addition to adult specimens, also included juvenile stages.

The authors of the present paper were interested in the following problems:

– whether and in what respect the alimentary canal microflora of juvenile forms differs from that of the adult ones,
– whether the composition of the alimentary canal microflora of juvenile and adult specimens of polyphages depends on the quality of food.

The species *A. coleoptrata* (L.), known to be polyphagous, was chosen for study on account of

— its great relative abundance and biomass in some soils,
— its being readily grown in laboratory on various foods,
— its living juvenile and adult forms being easy to determine.

2. Ecological requirements of *Achipteria coleoptrata*

2.1. Occurrence

The species was found in moss and litter in different forest associations (Willmann 1931, van der Hammen 1952, Kiełczewski 1957, Rajski 1961) and on meadows (Rajski 1961). Van der Hammen (1952) observed abundant occurrence of *A. coleoptrata* in the forest litter of *Salix repens* and in a birch forest on sandy dunes, whereas Rajski (1961) found it in large numbers in the association of French rye-grass (Arrhenatheretum elatioris); *A. coleoptrata* was dominant among mites, its dominance index "*D*" amounting to 15.5.

The results of the authors' own studies on the occurrence of *A. coleoptrata* in forest soils showed that it was fairly abundant in the association Querco-Carpinetum stachyetosum in Białowieża National Park and in the association Tilio-Carpinetum in the reserve "Las Piwnicki" near Toruń (table 1). No specimens of *A. coleoptrata* were found in the pine forests on the dunes in the island Wolin (Empetro nigri-Pinetum).

2.2. Vertical distribution in soil

Most authors report that *A. coleoptrata* occurs in raw humus. Rajski (1961, 1968) found it at the depth of 0—5 cm and its distribution in that layer was more or less uniform, nymphs in some summer samples occurring mainly in the humus horizon. The same author observed autumn migrations of nymphs in loose soils down to the depth of 12 cm.

The authors' own observations on the distribution of *A. coleoptrata* in the soil in spring are summarized in table 2. It follows from that table that *A. coleoptrata* occurred mainly in the litter *(AoL)* and detritus *(Aof)* horizons. It was also observed that juvenile specimens considerably prevailed over adults. It means that *A. coleoptrata* takes an active part in the conversion of slightly decayed litter fall.

Table 1. Occurrence of *Achipteria coleoptrata* in the forest soils (Data in thousand/m²)

Group of mites	Alluvial soil Fraxino-Ulmetum	Grey-brown soil Querco-Carpinetum	Muck soil Querco-Carpinetum	Rusty soil Querco-Carpinetum	Gley-podzolic soil Querco-Piceetum
A. coleoptrata	0.4	11.5	4.2	3.4	1.6
Other Oribatei	29.9	84.4	69.1	121.8	116.5
Dominance of *A. coleoptrata*	0.2	10.9	6.1	2.8	1.3

Table 2. Vertical distribution of *A. coleoptrata* in forest soils (Mean numbers in 100 cm³ samples)

Horizon	Alluvial soil		Grey-brown soil		Muck soil		Rusty soil		Gley-podzolic soil	
	adult	juv.	adult	juv.	adult	juv.	adult	juv.	adult	juv.
Aol	0.2	0.5	3.5	45.0	1.1	2.4	1.1	1.5	2.6	7.3
Aof	×	×	×	×	6.5	21.3	0.7	1.5	—	—
Aoh	—	0.5	3.2	3.9	1.5	0.4	—	—	—	—
A_1	—	—	—	—	—	—	—	—	—	—

2.3. Food requirements

A. coleoptrata is recognized by most authors as a non-specialised feeder. WALLWORK (1967) thinks that species of the genus *Achipteria* eat green plants, plant detritus, mosses, lichens, fungi and algae. The present authors' observations show that the mites fed on plant detritus develop slowly and those grown in laboratory show higher mortality. Bad results were also obtained with mites grown exclusively on mycelium. On algae (*Protococcus viridis* AGARDH.), on the other hand, *A. coleoptrata* developed well and mortality in laboratory grown mites was low. The mites developed best on plant detritus with an addition of algae.

To summarize the above data it can be concluded that *A. coleoptrata* occurs most abundantly in biologically active soils (grey-brown podzolic soils, fresh meadow soils), which are moderately humid and have an average content of organic substance and the reaction close to neutral. In these soils the species studied feeds mainly in the AoL and Aof humus horizons.

3. Material and methods

Mites for the investigations were collected in the reserve "Las Piwnicki" near Toruń (plant association Tilio-Carpinetum typicum). The microflora of the alimentary canal of juvenile and adult specimens feeding under natural conditions was examined immediately after collection in November 1973.

In January 1974 adult specimens from the same habitat were collected for further rearing under laboratory conditions. They were divided into two groups: one was fed on algae *(Protococcus viridis)*, the other on slightly decayed pine needles. The mites were kept in cultivation tubes with asbestos bottoms designed by SENICZAK (1972). The tubes were previously sterilized at a temperature of 160 °C. The food given to mites was taken from their natural habitat and was not sterilized. It was always given in some excess and coprolites were removed from the chambers every two days.

The microflora of the alimentary canal of adult specimens in both groups was examined after a month's rearing. Larvae obtained in the meantime were reared to the tritonymph stage, then used for study. Mites fed on pine needles developed much more slowly than those fed on algae, and the mortality of the former was higher than that of the latter.

The material from the alimentary canal of mites was treated by the method previously described (PRUSINKIEWICZ, STEFANIAK and SENICZAK 1975). This consists of embedding the proterosomal part of the mites in paraffin blocks and cutting off the opisthosoma with a sterile scalpel. Sterile collection of material directly from the alimentary canal is thereby achieved.

The method described was modified in that a small amount of sterile quartz sand was placed in a Petri dish and slightly wetted with sterile water. Then the material taken from the alimentary canal with a sterile needle was transferred to this "pulp", slightly ground and mixed with melted sterile agar medium, whose composition has been previously described (PRUSINKIEWICZ, STEFANIAK and SENICZAK 1975). In each experimental group of mites the material was taken from the alimentary canal of 10 specimens (from each separately).

Inoculated plates were incubated for 7—10 days at room temperature. All colonies were isolated and stored for further investigation. Applying commonly accepted methods and media, the following have been determined: the basic morphological characters of isolated bacterial strains (shape, Gram reaction, motility, production of endospores); some biochemical characters: catalase production, oxidation and fermentation of sugars in Hugh-Leifson medium (glucose, fructose, lactose, saccharose, maltose); the ability to hydrolyze gelatin, starch, chitin, pectins, cellulose and lignin. The ability to hydrolyze these compounds was also studied in isolated fungal strains.

Table 3. Colony counts of microorganisms isolated from the alimentary canal of *A. coleoptrata* fed

Specimen		from natural feeding ground				fed on pine
No.	Stage	Bacteria	Actino-mycetes	Yeasts	Fungi	Bacteria
1	adult	3	3	—	2	2
	juv.	2	4	—	1	1
2	adult	5	2	—	1	2
	juv.	3	4	—	2	2
3	adult	1	—	—	2	3
	juv.	4	2	—	2	2
4	adult	3	2	—	1	2
	juv.	2	6	—	2	2
5	adult	3	2	—	1	3
	juv.	4	5	—	—	1
6	adult	2	—	—	3	2
	juv.	3	5	—	1	4
7	adult	3	2	—	3	2
	juv.	4	4	—	1	3
8	adult	2	—	—	—	1
	juv.	3	3	—	1	4
9	adult	1	—	—	2	3
	juv.	4	5	—	2	2
10	adult	3	2	—	3	1
	juv.	2	4	—	3	3
average	adult	2.6	1.3	—	1.8	2.1
	juv.	3.1	4.2	—	1.5	2.4

Table 4. Some characteristics of microorganisms isolated from the alimentary canal of *A. coleoptrata*

Specimens' stage	Group of microorganisms	Shape	Gram reaction	Motility	Catalase activity	Gelatin proteolysis	Starch hydrolysis
Adult	Bacteria:						
		rods	n	—	—	—	+++
		rods	p	—	+++	+	—
		rods	p	—	+++	++	++
		rods	p	+	+++	—	++
		cocci	p	—	+	—	+
	Actinomycetes				++	++	+++
Juvenile	Bacteria:						
		rods	n	—	++	+	+++
		rods	n	—	+—	—	—
		cocci	p	—	—	—	—
	Actinomycetes				++	+++	+++

n = negative
p = positive

+++ = very strong
++ = strong
+ = weak
— = none

on different food

needles			fed on algae			
Actino-mycetes	Yeasts	Fungi	Bacteria	Actino-mycetes	Yeasts	Fungi
–	2	–	2	7	–	1
–	2	2	5	18	–	–
–	1	1	2	1	1	1
–	1	2	7	10	–	2
–	2	–	1	3	2	4
–	3	2	10	26	–	1
–	1	1	3	2	–	1
–	2	1	12	16	–	2
–	1	1	1	3	–	2
–	2	3	9	13	–	1
–	2	1	2	4	1	–
–	–	2	6	10	–	3
–	2	–	3	5	1	1
–	1	2	10	17	–	3
–	2	1	3	4	1	–
–	1	1	12	13	–	3
–	–	–	5	1	1	–
–	1	2	12	16	–	2
–	2	1	4	5	–	1
–	1	4	8	12	–	1
–	1.5	0.6	2.6	3.5	0.7	1.1
–	1.4	2.1	9.1	15.1	–	1.7

feeding in natural ground

Decomposition of				Action on				
Chitin	Pectin	Cellulose	Lignin	Glucose	Fructose	Lactose	Saccha-rose	Maltose
–	–	–	–	ac	ac	ac	ac	ac
–	–	+	–	alk	–	alk	–	–
+	–	–	–	ac	ac	ac	ac	ac
–	–	–	–	ac	–	–	–	ac
–	–	–	–	–	–	–	–	–
–	–	+	+	–	–	–	–	–
–	–	–	–	ac	ac	ac	ac	ac
–	–	++	–	–	–	–	–	–
–	–	–	–	–	ac	–	–	–
–	–	++	+	–	–	–	–	–

ac = acid reaction
alk = alkaline reaction
– = no colour change

Table 5. Some characteristics of microorganisms isolated from alimentary canal of *A. coleoptrata*

Specimens' stage	Group of microorganisms	Shape	Gram reaction	Motility	Catalase activity	Gelatin proteolysis	Starch hydrolysis
Adult	Bacteria:						
		rods	n	–	++	–	–
		cocci	p	–	+	–	+
		cocci	p	–	–	–	–
		cocci	p	–	+++	+	–
		cocci	p	–	++	–	–
	Yeasts:				+++	–	–
Juvenile	Bacteria:						
		rods	p	–	+++	+	++
		cocci	p	–	+++	–	–
		cocci	p	–	–	–	–
	Yeasts:				+++	+++	+++
	Yeasts:				+	–	–

n = negative
p = positive

+++ = very strong
++ = strong
+ = weak
– = none

Table 6. Some characteristics of microorganisms isolated from alimentary canal of *A. coleoptrata*

Specimens' stage	Group of microorganisms	Shape	Gram reaction	Motility	Catalase activity	Gelatin proteolysis	Starch hydrolysis
Adult	Bacteria:	rods	n	–	++	–	–
		cocci	n	–	+++	–	–
	Actinomycetes				++	++	++
	Yeasts				+++	–	+
Juvenile	Bacteria:	rods	n	–	–	–	+++
		rods	n	–	++	+	+++
		rods	n	–	++	–	–
		rods	n	–	–	–	+
		rods	n	–	+++	–	+
		rods	p	–	+++	+	–
		rods	p	–	+++	+	+
		rods	v	–	+++	–	+
		cocci	p	–	–	–	–
		cocci	p	–	+++	+	–
		cocci	p	–	+++	–	+++
	Actinomycetes				++	+++	+++

n = negative
p = positive
v = variable

+++ = very strong
++ = strong
+ = weak
– = none

fed on pine needles

Decomposition of				Action on				
Chitin	Pectin	Cellulose	Lignin	Glucose	Fructose	Lactose	Saccha-rose	Maltose
–	–	++	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–
–	–	–	–	–	ac	–	–	–
–	–	–	–	ac	ac	ac	–	ac
–	–	–	–	alk	–	alk	alk	–
–	+	+	–	ac	–	ac	ac	–
–	–	+	–	ac	–	–	–	–
–	–	–	–	–	–	–	–	–
–	–	–	–	–	ac	–	–	–
+	+	–	–	ac	ac	ac	ac	ac
–	–	+	–	–	–	–	–	–

ac = acid reaction
alk = alkaline reaction
– = no colour change

fed on algae

Decomposition of				Action on				
Chitin	Pectin	Cellulose	Lignin	Glucose	Fructose	Lactose	Saccha-rose	Maltose
–	–	++	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–
–	–	+	+	–	–	–	–	–
–	+	+	–	ac	ac	ac	ac	ac
–	–	–	–	ac	ac	ac	ac	ac
–	–	–	–	ac	ac	ac	ac	ac
–	–	++	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–
++	–	–	–	ac	ac	–	–	–
–	–	+	–	ac	ac	–	–	–
–	–	+	–	ac	ac	–	ac	ac
–	–	–	–	ac	ac	ac	ac	ac
–	–	–	–	–	–	–	–	–
–	–	–	–	ac	ac	ac	–	ac
–	+	–	–	ac	–	–	–	–
–	–	++	+	–	–	–	–	–

ac = acid reaction
alk = alkaline reaction
– = no colour change

4. Results

The quantitative occurrence of microorganisms in the alimentary canal of *A. coleoptrata* is illustrated by table 3. The data also show the recurrence range of the results within the experimental groups and at the same time they reflect the differences in the microflora of mites taking different foods.

The results obtained show that the microflora of the alimentary canal of juvenile forms is more abundant than that of adult specimens. These differences were especially pronounced with reference to Actinomycetes. Juvenile forms collected in their natural habitat had 3 times as many Actinomycetes as adult specimens, and juvenile specimens fed on algae had 6 times as many as adults.

The microflora of the alimentary canal of juvenile forms was also more active than that of adult specimens, which is illustrated by tables 4, 5 and 6. This is best seen on the example of Actinomycetes isolated from the alimentary canal of juvenile forms, which showed a higher proteolytic and cellulolytic activity (table 4, 6) than those isolated from the alimentary canal of adult specimens.

The most abundant and varied microflora was isolated from the alimentary canal of specimens fed on algae, and the poorest from those fed on pine needles.

An interesting fact is the absence of yeast in specimens collected in natural habitat and the presence of these organisms in specimens grown in laboratory. It may, therefore, be assumed that yeast multiplied in the cultivation tubes. This assumption is supported by the fact that more yeast was isolated from specimens fed on pine needles, which reached the tritonymph stage after a period 2—3 times longer than the specimens fed on algae. In the latter, on the other hand, it was the Actinomycetes that dominated; they were considerably less abundant in specimens feeding in the natural habitat, and completely absent in specimens fed on pine needles.

The most varied fungal microflora was isolated from the alimentary canal of species feeding in the natural habitat (table 7). In juvenile specimens of both laboratory grown groups fungi of the genus *Cladosporium* dominated; this might have been connected with an increasing fungus development in the cultivation tubes.

Table 7. Frequency of fungi isolated from the alimentary canal of *A. coleoptrata* fed on different food

Genera of fungi	Specimens					
	from natural feeding ground		fed on pine needles		fed on algae	
	adult	juv.	adult	juv.	adult	juv.
Aspergillus spp.	+ + +	—	+	+	—	+
Cladosporium spp.	+	+	+	+ + +	—	+ + +
Mortierella spp.	+	+	—	—	—	—
Penicillium spp.	+	+	—	—	—	—
Trichoderma spp.	+	+	+	—	+	—

5. Discussion and conclusions

It is known that *A. coleoptrata* occurs in large numbers in many biotopes, and that the number of its juvenile specimens often equals or even exceeds that of the adult. In the forest soil it occurs mainly in raw humus, the majority of specimens feeding in the litter *(AoL)* and detritus *(Aof)* horizons, i.e. in a slightly decayed layer of litter fall.

From observations on its feeding habits it follows that juvenile forms eat more than adult specimens. The results of BERTHET (1963) also show that 70% of the general metabolism of mites is contributed by their juvenile stages. According to our observations the microflora of the alimentary canal of juvenile forms is also richer and more active than that of adult specimens.

The results of the present work indicate that specimens feeding more intensively (mites fed on algae) have a richer and more active microflora than those feeding poorly (mites fed on pine needles). The former developed quickly and showed lower mortality, whereas the latter developed slowly and showed high mortality. It may, therefore, be assumed that a richer and more active microflora of the alimentary canal stimulates the activity of mites and at the same time promotes their more rapid development.

From the alimentary canals of juvenile and adult specimens of *A. coleoptrata* was isolated a fairly rich bacterial flora, active in the decomposition of carbohydrates and proteins. It is difficult to say now to what extent this microflora is symbiotic and to what degree it is accidental, depending on the kind of the food taken. However, it is important that this microflora takes part in the decomposition of organic substance eaten by mites and makes it possible or easier for them to digest such substances as cellulose, lignin or chitin.

Speaking of the role of mites, especially of their juvenile forms in the decomposition of organic substances in soil, it is difficult to omit the abundant and active microflora of the alimentary canal. Participating in the decomposition of the food taken in by mites, it stimulates the activity of mites in the environment.

The conclusions which follow from the present paper can be summarized in the following items: (1) The microflora of the alimentary canal of juvenile forms of *A. coleoptrata* is more abundant and varied than that of adult specimens. (2) The microflora of the alimentary canal of juvenile forms of *A. coleoptrata* is more active than that of adult specimens. (3) The microflora of the alimentary canal of juvenile and adult specimens of the species studied depends on the kind of food taken. The food stimulating the development of mites also activates the microflora of their alimentary canal.

6. Acknowledgements

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7. Summary · Zusammenfassung

In earlier studies of the microflora of the alimentary canal in moss-mites only adult individuals were taken into account (Prusinkiewicz, Stefaniak and Seniczak 1957).

Since, however, it is the juvenile forms that are considered more important in the transformations of organic substance in soil the authors undertook microbiological studies including not only adult but also juvenile stages.

The polyphagous species *A. coleoptrata* was chosen for study. Material from the alimentary canals of juvenile and adult individuals was taken by a method previously worked out (Prusinkiewicz, Stefaniak and Seniczak 1975). It consists in cutting off with a sterile scalpel the opisthosoma of mites with their proterosomal parts embedded in paraffin blocks, thus making it possible to take material directly from the alimentary canal under sterile conditions. The material was transferred with a sterile needle to a pulp made up of sterile quartz sand and water, slightly ground and mixed with melted sterile agar medium, whose composition was given in a previous paper (Prusinkiewicz, Stefaniak and Seniczak).

The media inoculated with the material from the alimentary canal were incubated for 10 days at room temperature, then the colonies were isolated. Applying the usual methods and media some data were obtained on the basic morphological characters of isolated bacterial strains, some of their biochemical characters and their ability to hydrolyze protein.

Here are the main conclusions following from the paper:

1. The microflora of the alimentary canal of juvenile forms of *A. coleoptrata* is more abundant and varied than that of adult specimens.
2. The microflora of the alimentary canal of juvenile forms of *A. coleoptrata* is more active than that of adult specimens.
3. The microflora of the alimentary canal of juvenile and adult specimens of the species studied depends on the kind of food taken. The food stimulating the development of mites also activates the microflora of their alimentary canal.

Bisher untersuchten wir die Mikroflora des Verdauungskanals von erwachsenen Moosmilben (Prusinkiewicz, Stefaniak und Seniczak 1975).

Die Tatsache, daß juvenile Moosmilben bei der Zersetzung der organischen Substanz im Boden als die wichtigeren angesehen werden, veranlaßte die Verfasser zu mikrobiologischen Untersuchungen, in welchen neben erwachsenen Individuen auch juvenile Moosmilbenformen berücksichtigt wurden. Als Untersuchungsobjekt dienten Tiere der polyphagen Art *A. coleoptrata*. Das Material für mikrobiologische Untersuchungen wurde nach der vorher beschriebenen Methode entnommen (Prusinkiewicz, Stefaniak und Seniczak 1975). Dabei wurden die Milben in Paraffinblöckchen eingebettet, ihr Epistosome mit einem sterilisierten Skalpell entfernt, der Körperinhalt mit einer sterilisierten Nadel in sterilen feuchten Quarzsand übertragen und eingemischt. Der beimpfte Quarzsand wurde sodann mit Nähragar übergossen (s. Prusinkiewicz, Stefaniak und Seniczak 1975). Die beimpften Nährböden wurden 10 Tage lang bei Zimmertemperatur inkubiert und die Kolonien nachher isoliert.

Bei Anwendung der allgemeinen Methoden und Nährböden bestimmten wir die morphologischen und biochemischen Merkmale sowie die Eiweiß-Hydrolysenfähigkeit der Bakterienstamme.

Aus der Arbeit ergeben sich folgende Schlußfolgerungen:

1. Die Mikroflora des Verdauungskanals der juvenilen Formen von *A. coleoptrata* ist zahlreicher und unterschiedlicher als bei erwachsenen Individuen.
2. Die Mikroflora des Verdauungskanals der juvenilen Formen von *A. coleoptrata* ist aktiver als bei erwachsenen Individuen.
3. Die Mikroflora des Verdauungskanals der jugendlichen und erwachsenen Individuen der untersuchten Art ist von der Qualität der entnommenen Nahrung abhängig. Eine Nahrung, die die Entwicklung der Milben ermöglicht, aktiviert auch die Mikroflora im Verdauungskanal.

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